Bioecology and Vectorial Capacity of *Aedes albopictus* (Diptera: Culicidae) in Macao, China, in Relation to Dengue Virus Transmission

Author(s): A. Paulo G. Almeida, Susana S. S G. Baptista, Carla A. G C C. Sousa, M. Teresa L M. Novo, Helena C. Ramos, Nicholas A. Panella, Marvin Godsey, M. João Simões, M. Luisa Anselmo, Nicholas Komar, Carl J. Mitchell, and Henrique Ribeiro

Source: Journal of Medical Entomology, 42(3):419-428.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0022-2585(2005)042[0419:BAVCOA]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1603/0022-2585%282005%29042%5B0419%3ABAVCOA%5D2.0.CO%3B2

Bioecology and Vectorial Capacity of *Aedes albopictus* (Diptera: Culicidae) in Macao, China, in Relation to Dengue Virus Transmission

A. PAULO G. ALMEIDA,[1] SUSANA S.S.G. BAPTISTA,[1] CARLA A.G.C.C. SOUSA,[1] M. TERESA L. M. NOVO,[1] HELENA C. RAMOS,[1,2] NICHOLAS A. PANELLA,[3] MARVIN GODSEY,[3] M. JOÃO SIMÕES,[1] M. LUISA ANSELMO,[1] NICHOLAS KOMAR,[3] CARL J. MITCHELL,[3,4] AND HENRIQUE RIBEIRO[1]

J. Med. Entomol. 42(3): 419–428 (2005)

ABSTRACT Until 2001, the Chinese Territory of Macao had not registered any autochthonous dengue cases, despite the abundance of *Aedes albopictus* (Skuse), a known vector. This work describes a bioecological characterization of the local *Ae. albopictus* adult population, with the purpose of estimating the receptivity of Macao to dengue introduction. In the wet seasons of 1997 and 1998 and the dry season of 1998, *Ae. albopictus* was the most abundant human-biting mosquito. Daily biting rates of 314 mosquitoes per person were recorded in the wet season with a reduction to 94 in the dry season. *Ae. albopictus* was mainly exophagic and exophilic and had a human blood index of 44%. The parity rate of field-collected mosquitoes was 57%. Daily survival rate ranged from 91 to 97%. Estimates of vectorial capacity ranged from 144 to 880, depending on what parameter values were used. These estimates indicated a great receptivity for the introduction of dengue viruses, as the 2001 outbreak came to prove.

KEY WORDS *Aedes albopictus*, China, dengue, bioecology

MACAO, SITUATED CLOSE TO Hong Kong in southeastern China (22° 14′ N, 113° 35′ E), borders endemic areas for dengue viruses (Flaviviridae: *Flavivirus*), but never registered transmission of this virus until 2001, despite harboring *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894), one of the most competent dengue vectors (Easton 1994). In 2001, an outbreak with 1,418 cases occurred (Lim 2002, Maia Trindade 2002).

Previous surveys in Macao revealed *Ae. albopictus* as one of the most abundant mosquitoes since its first record in 1990 (Ramos 1990, 1994; Ramos et al. 1997). *Ae. albopictus* is originally a Southeast Asian mosquito, and in recent years it has become cosmopolitan due to its introduction by human worldwide (Knudsen 1995). Macao has a tropical moist, or monsoon climate that favors a high quantity and diversity of mosquito habitats, particularly for *Ae. albopictus* (Ramos et al. 1997, Novo 2000). The common dengue vector *Ae.* (*Stegomyia*) *aegypti* (Linnaeus, 1762) is absent from Macao, having only been recorded once, in 1921 (Leitão 1921; Ramos 1990, 1994; Ramos et al. 1997).

Ae. albopictus has been the focus of entomologists, virologists, and public health workers because it is a highly competent vector for several arboviruses, including the four dengue virus serotypes (Hawley 1988, Mitchell 1995b). Dengue viruses are endemic in countries surrounding Macao, such as Thailand, Vietnam, and mainland China (WHO 2002). Diseases caused by dengue viruses are reportable in most countries, although perhaps only a small proportion of cases are reported to World health Organization (WHO 2002). In Southeast Asia, dengue hemorrhagic fever (DHF) cases have been increasing from an annual rate of <10,000 in the 1950s and 1960s to >200,000 in the 1990s (Gibbons and Vaughn 2002). It is estimated that each year 50 million cases of dengue fever with 500,000 cases of DHF, occur around the world with at least 12,000 deaths (WHO 2002).

The absence of autochthonous dengue transmission in Macao was puzzling, given the proximity of dengue endemic areas and the abundance of *Ae. albopictus.* The construction of an international airport in Macao, in 1996, with the consequent rise in movement of people to and from neighboring dengue endemic countries, increased the risk of outbreaks considerably. To better understand the risk of dengue transmission in Macao, we characterized the bioecology of the adult population of *Ae. albopictus* in 1997–1998, with the objective of estimating Macao's receptivity to

[1] Medical Entomology Unit, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 96, 1349-008 Lisboa, Portugal.

[2] Instituto de Investigação Científica Tropical, Rua da Junqueira 14, 1300-343 Lisboa, Portugal.

[3] Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, P.O. Box 2087, Fort Collins CO 80521.

[4] Deceased.

dengue, before any locally transmitted dengue fever cases had been reported.

Materials and Methods

Study Area. Macao (population 435,000) has an area of 21 km^2 situated in the Pearl River delta. The study area involved the Macao peninsula (7.5 km^2), on which the city is located, and where 90% of the population lives, and two islands, Taipa (5.8 km^2) and Coloane (7.8 km^2). Mean annual temperature is 20°C, mean annual relative humidity ranges between 75 and 90%, and total annual precipitation is 1,000–2,000 mm.

The terrain is predominantly granitic with considerable alluvial deposition that has increased its area as well as artificial drainage that has permitted increased construction, particularly in the city of Macao and in Taipa. Macao is hilly (maximum altitude 160 m above sea level) with scattered luxuriant gardens and forested areas on the islands (Dias et al. 1994). The vegetation is mainly oriental evergreen forest, with some shrubby and mangrove swamp areas (CMI and IICT 1991, Dias et al. 1994, Hao 1997).

Mosquito Collections. Mosquito collections occurred during three field trips, two at the end of the rainy season (September–October, 1997 and 1998) and one at the end of the dry season (March 1998). The wet season, from April to September, is characterized by an average monthly precipitation of 150–200 mm and mean temperature of 25°C, whereas during the dry season, from October to March, monthly precipitation averages 50–100 mm, with a mean temperature of 15–20°C.

Nine collection sites were selected in the city of Macao, mainly gardens or parks, and one cemetery. Seven sites were chosen in Taipa, including cemeteries, parks, farms, a tire factory, and an abandoned factory; and eight sites were chosen in Coloane, including Seac Pai Van and Cheoc Van parks, large villas, youth hostels, a riding school, and a temple.

Human bait collections were performed using battery-powered hand aspirators in Seac Pai Van Park in Coloane, to establish the daily activity cycle of mosquitoes and the human daily biting rate of *Ae. albopictus.* During the wet season, 11 collection periods ranging from 5 to 24 h each were performed by one to three teams of two people, thus covering all hours of the day. Each hour was covered by at least four such collection periods. In the dry season, three teams of two people covered a period of 13 h, 0600–1900 hours simultaneously.

To understand the exophagic and endophagic behavior of *Ae. albopictus*, human bait collections were carried out simultaneously indoors and outdoors, for a period of 2 h in the morning (0630–0830 hours) and evening twilight (1730–1930 hours), after complete removal of indoor resting mosquitoes. Human biting rate per person and hour were calculated for each situation. Three collection sites were selected: a greenhouse in Lou Lim Ioc garden in Macao city, a human residence at a goose farm on Taipa Island, and the guard shack at Seac Pai Van Park on Coloane Island.

Indoor resting mosquitoes were collected for the endophily/exophily characterization and determination of bloodmeal origin of *Ae. albopictus.* Outdoor mosquitoes resting in the vegetation and natural shelters were collected with a backpack aspirator (Clark et al. 1994). CDC light traps baited with CO_2 (Newhouse et al. 1966) were used for nocturnal collections in most of the above-mentioned locations.

Mosquito Identification and Processing. Mosquitoes were frozen after collection and later identified on chilled tables. *Ae. albopictus* females were divided in two main groups: those for arbovirus detection, which were frozen in liquid nitrogen, and those for parity determination, which were frozen at −70°C. The latter were dissected, and their ovaries removed and classified according to Christophers and Mer stages (Forattini 1965) and examined by the method of Detinova (1963) for the observation of the tracheole coils and classified as nulliparous or parous.

Freshly blood-fed *Ae. albopictus* females aspirated from vegetation and indoor resting sites were dissected and their midguts squashed onto Whatman no. 1 filter paper. Papers were dried and stored with silica gel at room temperature.

Mosquito Bloodmeal Source Identification. Mosquito bloodmeal source was identified using a two-site enzyme linked immunosorbent assay (Simões et al. 1995). Antibodies against human, dog, pig, chicken, goat/sheep, and rat/mouse immunoglobulin G were tested. Blood samples of known vertebrates served as controls. Four positive controls (homologous blood) and 12 negative controls (heterologous blood) were applied to every 96-well microtiter plate. Absorbance values were read at 492 nm, and the cut-off values of the assays were calculated as the mean plus three times the standard deviation of the negative controls.

Screening of Field-Caught Mosquitoes for Arboviruses. Mosquitoes were pooled according to species, collection method, and location, up to 50 individuals per pool. These pools were macerated in 2 ml of physiological buffer containing antibiotics and centrifuged. A 0.1-ml aliquot of each clarified supernatant was inoculated in duplicate onto Vero cell monolayers in six-well plates (Costar, Cambridge, MA), incubated at 37°C for 1 h, and then cultured under a 3 ml of 0.5% agarose overlay for up to 10 d. Plates were stained with neutral red for visualization of viral plaques by adding a second agarose overlay containing neutral red either on day 2 postinoculation (first replicate) or day 5 postinoculation (second replicate).

Endophagic and Endophilic Indexes. Endophagic and endophilic indexes (Ribeiro and Janz 1990) were determined as follows.

The endophagic index (ENGI) is obtained by the formula

$$\mathrm{ENGI} = \frac{\mathrm{IMBR}}{\mathrm{OMBR} + \mathrm{IMBR}}$$

where IMBR is the indoor human-biting rate and OMBR is the outdoor human-biting rate. The endophilic index (ENLI) is given by the formula

$$\mathrm{ENLI} = \frac{\mathrm{IRDG}}{\mathrm{IMBR}\ (i-1)j}$$

where IMBR is the indoor human-biting rate; IRDG is the indoor resting density of gravids (including semigravids); i represents the length in days of the gonotrophic cycle, lessened by 1 to exclude just fed females; and j corrects for mortality, being a function of i and of the daily survival rate (p), as follows:

$$j = \sum_{k=1}^{i-1} p^k$$

Establishment of Laboratory Colony of *Ae. albopictus*. For the estimation of several biological parameters of *Ae. albopictus*, particularly those necessary for the determination of vectorial capacity, a colony was established from immature stages collected in the field. Insectary conditions were set to mimic conditions during the wet season, with a temperature of 25°C, 80–90% RH, and a photoperiod of 16:8 (L:D). Emerging adult female mosquitoes were kept individually in 2 by 2 by 5-cm cages, after staying with males for 24 h. These mosquitoes were offered the possibility of blood feeding on human blood for 10 min every day, supplied with sugar solution and a wet filter paper for egg laying. Bloodmeals, egg batches, and deaths were recorded daily for 21 d. Thus, the length of the first (i_0) and subsequent (i) gonotrophic cycles were recorded, as well as the frequency of feeding. Life tables were constructed and the daily cumulative chance of survival was calculated according to Kirkwood (1988).

Daily survival rate (p) of *Ae. albopictus* was calculated by the formula $\sqrt[i_0]{M}$, where i_0 is the length of the first gonotrophic cycle and M is the parity rate (Davidson 1954), or by the formula $\sqrt[d]{P}$, in which d is the number of days that the study lasted and P the proportion of females alive by the end of that period.

Vectorial capacity, C (the number of new potentially infectious mosquito bites for each day a person is infectious) of the Macao *Ae. albopictus* population was calculated as

$$C = \frac{m\ a^2 p^n}{-\log_e p}$$

in which ma is the human daily biting rate, with m as the mosquito density in relation to humans and a as the human biting habit; p is the daily survival rate of the mosquitoes, and n is the extrinsic incubation period (in days) of dengue viruses in *Ae. albopictus* (Garrett-Jones 1964).

Statistical Analysis. Means for all observations were calculated and compared by paired or unpaired Student's *t*-tests. Feeding frequency and daily survival data were compared by Mantel–Haenszel χ^2 (Kirkwood 1988). Statistical tests were performed using the statistical package Nanostat (London School of Hygiene and Tropical Medicine, University of London, London, England).

Results

Mosquito Abundance. In total, 10,909 mosquitoes were collected, with similar effort expended during both dry (660 collecting hours) and wet seasons (684 collecting hours). However, the yield was higher in the wet season (11 mosquitoes/h) than in the dry season (five mosquitoes/h). *Ae. albopictus* was the most abundant mosquito in Macao, with 48% of all mosquitoes caught being *Ae. albopictus* females (5,237) and 29% *Ae. albopictus* males (3,131).

The other species caught were *Anopheles* (*Anopheles*) *sinensis* Wiedemann, 1828; *Aedes* (*Stegomyia*) *w-albus* (Theobald 1905); *Armigeres* (*Armigeres*) *subalbatus* (Coquillett, 1898); *Armigeres* (*Leicesteria*) *magnus* (Theobald 1908); *Coquillettidia* (*Coquillettidia*) *crassipes* Van der Wulp, 1881; *Culex* (*Culex*) *bitaeniorhynchus* Giles, 1901, *Culex* (*Culex*) *quinquefasciatus* Say, 1823; *Culex* (*Culex*) *sitiens* Wiedemann, 1828; *Culex* (*Culex*) *tritaeniorhynchus* Giles, 1901; *Culex* (*Culex*) *vagans* Wiedemann, 1828; *Culex* (*Culex*) *vishnui* Theobald, 1901; *Culex* (*Culiciomyia*) *pallidothorax* Theobald, 1905; *Culex* (*Lophoceraomyia*) *rubithoracis* Leicester 1908; *Culex* (*Lophoceraomyia*) *sumatranus* Brug, 1931; *Mansonia* (*Mansonioides*) *uniformis* (Theobald 1901); *Uranotaenia* (*Uranotaenia*) *edwardsi* Barraud, 1926; and *Uranotaenia* (*Uranotaenia*) *macfarlanei* Edwards, 1914, corresponding to 46% of the 37 mosquito species known from Macao (Ramos et al. 2000, 2002).

As to the collection methods, human-baited collections were carried out for 146 h, yielding 52 mosquitoes/person/h, of which 94% were *Ae. albopictus.* CDC light traps baited with CO_2, used for 1,188 hours, attracted 1.8 mosquitoes/h, of which only 3% were *Ae. albopictus.* Despite having been used for the least time, 11 h, backpack aspirator collections from vegetation gave the highest yield, 110 mosquitoes/h, of which 98% were *Ae. albopictus.* Indoor resting collections were least efficient.

The yield of the human-baited collections increased from the dry to the wet season, from 19 to 60 mosquitoes/person/h, with *Ae. albopictus* maintaining a share of 94%. On the contrary, the yield of CDC traps decreased from the dry to the wet season, from 2.9 to 0.5 mosquitoes/h, respectively, mainly due to the decrease of species other than *Ae. albopictus.* However, the yield from backpack aspiration was not significantly changed between seasons. *Ae. albopictus,* compared with remaining species, has a similar proportion between seasons, in both human baited (93% dry, 94% wet) and backpack aspiration (98% in both seasons) collections. However, in CDC collections, *Ae. albopictus* has a higher proportion in the wet season compared with the dry season (17% versus 0.01%, χ^2. = 196.8, df = 1, P = 0.0000). Considering all collection methods, *Ae. albopictus* accounted for 40% of the mosquitoes collected in the dry season and 91% in the wet season (χ^2. = 3310, df = 1, P = 0.0000). The proportion

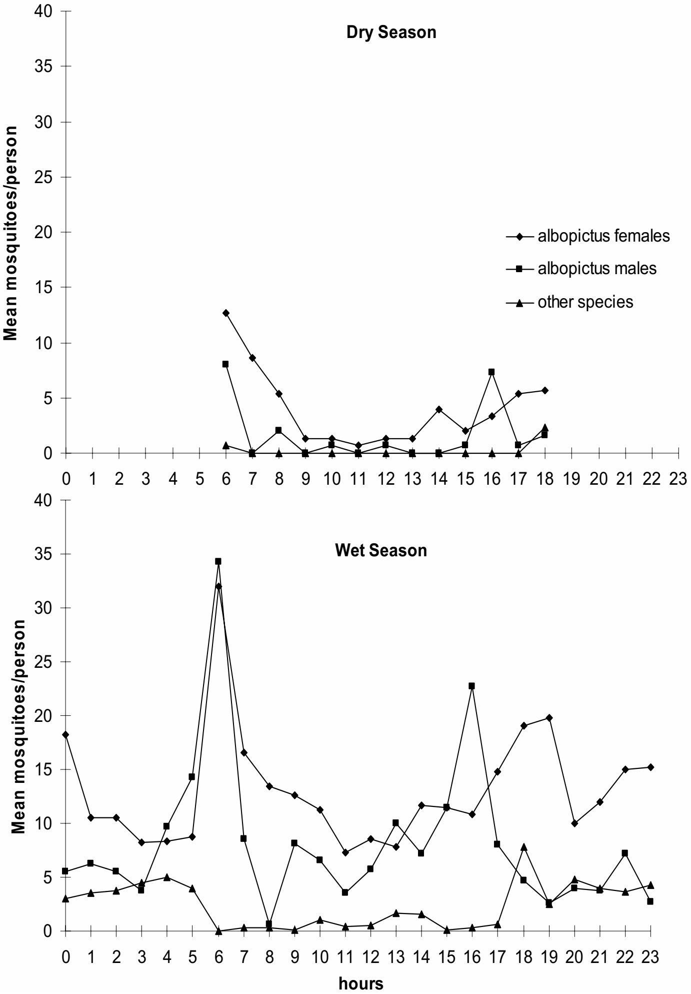

Fig. 1. Daily activity cycle of mosquitoes in Macao, China, in the dry and wet seasons. *Ae. albopictus* females and males and mosquitoes belonging to other species caught by human-baited landing catches.

of females *Ae. albopictus* only revealed differences between the human-baited collections with a decrease from the dry, 72%, to the wet season, 62% (χ^2. $= 21.9$, df $= 1$, $P = 0.0000$), which was diluted in the total collecting methods, not revealing differences between seasons, 65% in the dry and 62% in the wet season.

Taipa was the region with the highest mosquito abundance, with 17.3 mosquitoes/person/h in the crepuscular human-baited collections. Coloane was next with 7.8 mosquitoes/person/h, and finally the city of Macao with 2.8 mosquitoes/person/h, with most mosquitoes collected in the gardens.

Daily Activity Cycle. The daily activity cycle of human biting mosquitoes in Macao was assessed through human-baited landing. In both seasons, *Ae. albopictus* was the most abundant human-biting mosquito (Student–Fisher *t*, $P < 0.01$) (Fig. 1).

Collections performed in the wet season revealed two activity peaks for *Ae. albopictus*, coinciding with crepuscular dawn, 0600–0800 hours, and dusk, 1800–2000 hours, with an average of 48.6 female mosquitoes in the morning peak and 38.9 female mosquitoes in the evening peak. Between these peaks, the activity was lower but never null. The activity profile of the coinciding 13-h period measured in the dry season revealed a similar pattern, with identical peaks (Fig. 1), although with much lower densities (Student–Fisher *t*, $P < 0.01$). Male *Ae. albopictus* had coincident morning peaks, but their evening peaks preceded those of the females by $\approx$1 h (1600–1700 hours), in both wet and dry seasons. Frequently, male swarms preceded female biting activity, with copulation attempted during blood-feeding of females. Couples copulating in flight were often observed. Although similar in the peaks, male densities were lower than female densities for the remaining observation periods. The other species showed a peak at 1800 hours of approximately eight mosquitoes/person/h, after which a low but constant activity was kept through the night and ceased at dawn, again similar in both seasons for the

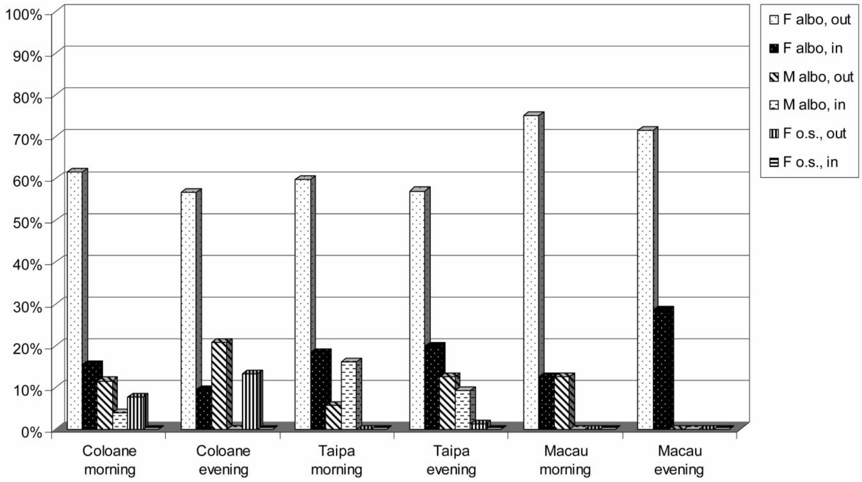

Fig. 2. Human baited mosquito collections, simultaneously outdoors (out) and indoors (in), for the morning (0630–0830 hours) and evening (1730–1930 hours) periods, in Coloane, Taipa, and Macao peninsula, represented as percentage of mean mosquitoes/person/h for each location, and period. F.albo, female *Ae. albopictus*; M.albo, male *Ae. albopictus*; F.o.s., females of other species.

coinciding observation period (Student–Fisher t, $P >$ 0.05).

Ae. albopictus were thus the most important of the human-biting mosquitoes in Macao, with a mean daily human biting rate of 314 females/person/d in the wet season. Because the observations carried out in the dry season had identical trends to the corresponding period in the wet season, we estimated a human biting rate of 94 females/person/d.

Exophagic and Endophagic Behavior. Human-baited collections indoors and outdoors confirmed *Ae. albopictus* as the most abundant anthropophilic mosquito, ranging from 87 to 100% of the collections (*Ae. albopictus* both sexes, in relation to total collections) (Fig. 2). The proportion of *Ae. albopictus* females feeding outdoors ranged from 71 to 86%, whereas 14 to 29% fed indoors (paired t-test $t = -3.5$, $P < 0.05$). The predominantly exophagic behavior was confirmed by a low endophagy index, which ranged from 0.17 to 0.25.

Exophilic and Endophilic Behavior. We collected only 11 *Ae. albopictus* females indoors, 10 in one house in Taipa, and one in the horse riding school in Coloane. Only two (18%) were gravid. Therefore, the endophily index could only be determined in Taipa, yielding a value of 0.09, which pointed clearly to exophilic behavior.

Feeding Preferences. In total, 52 bloodmeals were examined (Table 1), which included earlier collections from Taipa and Coloane (Ramos et al. 1997). Humans seem to be the favorite hosts for *Ae. albopictus* as indicated by a human blood index of 0.37 for Coloane specimens and 0.64 for Taipa specimens (Table 1). In Taipa, the secondary origin of the bloodmeals was dogs (0.15), and a large proportion remained unknown (0.23). In Coloane, many other hosts, such as chickens (0.32), dogs (0.16), horses (0.08) and goats (0.05), provided bloodmeals to *Ae. albopictus.* Mixed bloodmeals occurred at a low rate (0.05). Bloodmeals tested were negative to cow, rabbit, pig, and rat blood. Overall, anthropophily or the human blood index in Coloane and Taipa is ≈0.44.

Parous Rate. For the determination of the parous rate of *Ae. albopictus,* 176 field-caught *Ae. albopictus* females were dissected, of which 93 were parous (52.8%). However, if we consider those in stage V as parous (Hamon 1963), parous rate rises to 56.8%.

Screening of Field-Caught Mosquitoes for Arboviruses. In total, 4,150 mosquitoes (2,268 *Ae. albopictus,* four *An. sinensis,* 18 *Ar. subalbatus,* two *Ar. magnus,* 12 *Cq. crassipes,* 39 *Culex quinquefasciatus* (Say), 1,651 *Cx. sitiens,* and eight *M. uniformis*) were screened for arboviruses by plaque assay, in 131 pools, and no cytopathic effect was detected.

Feeding Frequency and Gonotrophic Cycle. The remaining bioecological parameters of *Ae. albopictus* had to be studied based on the observations of a laboratory colony, established for that purpose. *Ae. albopictus* females were monitored for 21 d.

The number of bloodmeals taken by colony females ($n = 54$) allowed us to determine the daily feeding

Table 1. Blood preferences of female *Ae. albopictus*, as tested by ELISA, for the islands of Taipa ($n = 14$) and Coloane ($n = 38$)

Blood source	Taipa	Coloane	Combined
Human	64 (9)	37 (14[a])	44 (23)
Chicken		32 (12[a])	23 (12)
Dog	15 (2)	16 (6)	15 (8)
Horse		7.9 (3)	5.8 (3)
Goat		5.3 (2[a])	3.8 (2)
Negative/other	23 (3)	2.6 (1)	7.7 (4)

Percentages of positive results are followed by actual positive samples for each type of host blood in parentheses.

[a] Mixed bloodmeals: one with human/goat blood, and one with human/chicken blood.

Table 2. Computations of the vectorial capacity of *Ae. albopictus* in Macao for dengue viruses (1997–1998) for a 10-d extrinsic incubation period (Hawley 1988)

Mean human-biting rate (ma)	Wet season (314 mosquitoes/person/d) Dry season (94 mosquitoes/person/d [estimate])	
Human blood index (HBI)	0.44	
Feeding frequency (f)	0.31 bloodmeal/d	
Human biting habit (a)	0.138	
a = HBI × f		
Duration of first gonotrophic cycle (i_0)	10.7 d	
Parous rate (M)	0.568	
Daily survival rate (p)	from parous rate 0.948	from proportion alive 0.966
Vectorial capacity (C)		
Wet season	482	880
Dry season	144	263

frequency which was 0.31 bloodmeals per days (262 bloodmeals in 839 female-days). This means roughly a bloodmeal every 3.2 d. The proportion of females that blood fed was 98%; however, only 28% laid eggs, possibly due to a low insemination rate in females exposed to males for only 24 h. The majority of females (90%) had taken their first bloodmeal by the end of the third day.

Females that laid eggs in the laboratory had a first gonotrophic cycle (i_0) of 10.67 ± 4.75 d (mean ± SD) and the subsequent mean gonotrophic cycles of 4.14 ± 1.77 d, significantly shorter (unpaired *t*-test, $t = 4.385$, $P = 0.0001$).

Daily Survival Rate (p). The p value was calculated using M = 0.568 and $i_0 = 10.67$ and the formula $p = \sqrt[i0]{M}$ (Davidson 1954), resulting in $P = 0.948$.

An alternative approach to calculating p used observations from the laboratory colony. The proportion of laboratory females alive at the end of the 21 d of observation, was 48.1%, then $p = \sqrt[21]{0.481} = 0.966$.

Vectorial Capacity. We calculated this value based on field parameters observed in the wet season, particularly the mean human biting rate, and on the colony of *Ae. albopictus.* The C value thus took two values, according to which method for estimating the daily survival rate was used. With p estimated on the parous rate, we calculate C = 482 (Table 2). However, survival rate based on the proportion of females that survive in the laboratory gave C = 880. The corresponding values for vectorial capacity in the dry season, with an estimated human daily biting rate of 94 mosquitoes/person/d, would be C = 144 and 263.

Discussion

Ae. albopictus was by far the most abundant mosquito in the territory of Macao, China, accounting for 77% of the total mosquitoes collected, confirming previous surveys (Ramos et al. 1997). Given its vector status for a variety of arboviruses, including dengue viruses, this mosquito should be considered a public health threat in Macao. Other mosquito species collected were present in low numbers, generally bite at night when most people are indoors and therefore protected from exposure to mosquitoes. Considering these mosquito species are not competent vectors of dengue viruses, they should not be considered important in terms of public health.

Ae. albopictus was also the most abundant human-biting mosquito, in either season, although abundance decreased in the dry season. In human-baited or backpack aspirator collections, 94 and 98%, respectively, of the mosquitoes caught were *Ae. albopictus.* The aspirator collections from vegetation would be expected to detect large numbers of nonhuman-biting mosquitoes if they were present. This method was also the most efficient for collecting *Ae. albopictus,* yielding 110 mosquitoes/h. This study indicates that human landing collections and aspirator collections from the vegetation are more efficient at capturing *Ae. albopictus* than are CO_2 baited CDC light traps. These failed to attract this species, as previously shown in earlier studies by Parsons et al. (1974), Freier and Francy (1991), and Jensen et al. (1994).

Of the three Macao regions, Taipa had the highest abundance of *Ae. albopictus* followed by Coloane, whereas the Macao peninsula had the lowest abundance. This is perhaps due to the lesser urbanization and higher number of larval habitats in Taipa and Coloane compared with the city (Novo 2000). The abundance of larval habitats in Taipa, particularly in the cemetery, where *Ae. albopictus* was the predominant species (Novo 2000), clearly explains the higher values. The Macao peninsula had the lowest abundance probably due to the high urbanization and corroborated by the finding of *Ae. albopictus* mainly in gardens. *Ae. albopictus* has been described as essentially a rural and suburban mosquito, which is regularly encountered in cities with vegetation (Hawley 1988).

Peak biting activity for *Ae. albopictus* was clearly bimodal, with morning and evening twilight peaks for both sexes and in both seasons, as observed elsewhere in Southeast Asia (Hawley 1988, Hassan et al. 1996). Contrary to observations in Singapore and the Philippines (Ho et al. 1971, Basio and Santos-Basio 1974), the dawn peak in Macao was higher than the evening peak. However, we observed activity during all 24 h. These observations were carried out in the highly forested Seac Pai Van Park in Coloane, which may have accounted for this continuous baseline activity. Less pronounced bimodal peaks have been reported in areas shielded with vegetation from the midday sun

(Macdonald and Traub 1960). We did not register a 1230-hour peak as in Malaysia (Hassan et al. 1996). Although *Ae. albopictus* reportedly rarely bites at night (Hawley 1988), we registered low but continuous biting activity throughout the night. This continuous activity may be due to strong attraction for human bait not normally present in forested habitat (Amerasinghe and Ariyasena 1991).

Humans were the most common bloodmeal source for *Ae. albopictus* in Macao, which further emphasizes its importance as a potential human pathogen vector. The elevated human blood index (HBI) observed on Taipa compared with Coloane is in agreement with the higher urbanization and higher human population density of the former. Human blood index determination is one of the most crucial parameters in the vectorial capacity model, and great care should be taken to avoid sampling bias (Garrett-Jones 1964, Garrett-Jones et al. 1980). Besides being small ($n = 52$) our collection of engorged specimens may have been biased as it resulted from indoor resting collections in human and animal dwellings, and no bloodfed mosquitoes were caught on the vegetation. However, similar anthropophily for this species has been reported previously (Hawley 1988).

Ae. albopictus is an opportunistic feeder upon most groups of vertebrates, including birds, reptiles, and amphibians, although preferring mammals (Colless 1959, Gould et al. 1970, Jumali et al. 1979, Savage et al. 1993, Niebylski et al. 1994). In Macao, we also observed opportunistic behavior of this species, because avian blood was detected in engorged females almost as frequently as human blood in Coloane, a forested island with few human inhabitants. *Ae. albopictus* is known to prefer outdoors (Rozeboom and Cabrera 1964, Gould et al. 1970, Chan 1971, Lambrecht 1971, Jumali et al. 1979). In Macao, *Ae. albopictus* fed preferentially outdoors and rested even less indoors, based on exophagy and endophagy and exophily and endophily indexes described by Ribeiro and Janz (1990).

About half of the *Ae. albopictus* female population from Macao were parous, which is very similar to that found in neighboring countries, such as Singapore (55%) or Thailand (61%), and within the range observed for this species in Asia, 18 to 68%, with a median of 55% (Hawley 1988). The parity rate from the colony females was only 28%, clearly not realistic and much lower than other laboratory studies showing 100% parity in a first cycle (Gubler and Bhattacharya 1971). Parity rate of a mosquito population is a critical factor in epidemiological entomology, because the biting rate increases in parous, older, and larger females (Xue and Barnard 1996). A high parous rate is also an indicator of longevity, or high daily survival rate (Davidson 1954). However, sampling method affects the parity rate. For example, the collection of indoor-resting mosquitoes is likely to yield gravid females of anopheline mosquitoes (Garrett-Jones and Shidrawi 1969). This was not the case with *Ae. albopictus* because our indoor resting collection was very unproductive and most mosquitoes for parity determination came from human-baited collections.

The length of the gonotrophic cycle is often calculated as the inverse of the daily feeding frequency. However, this only applies when there is one bloodmeal per gonotrophic cycle (Garrett-Jones and Shidrawi 1969, Klowden and Briegel 1994). This was clearly not our case because females in the laboratory averaged 3.4 bloodmeals per gonotrophic cycle. The length of the gonotrophic cycle of *Ae. albopictus* varies between 3–5 d in India, Vietnam, Singapore, and China (Hawley 1988). However, the length of the first gonotrophic cycle we measured in the laboratory was $\approx$10 d, clearly above previous reports of laboratory data (Gubler and Bhattacharya 1971, Hien 1976b). The reduced dimensions of the cages may have influenced the mosquitoes' behavior. Alternatively, some females may have needed a second engorgement to stimulate oviposition (Hawley 1988). However, the mean of all gonotrophic cycles we registered was 4.1 d, similar to 4.6 d found by Gubler and Bhattacharya (1971). Strain differences and our small sample size also may explain the divergence.

The length of the gonotrophic cycle is required to estimate the daily survival rate (Davidson 1954). We estimated a survival rate of 94% with the observed length of the gonotrophic cycle, but it would be slightly less, 91.8%, if we used the published length of the gonotrophic cycle, 4.6 d, and added 2 d from emergence to the first bloodmeal observed by us and others (Gubler and Bhattacharya 1971, Hien 1976a, Hawley 1988), as the length of the first gonotrophic cycle. Alternatively, if we consider the length of the subsequent gonotrophic cycles in our colony, 4.14 d, and add the 2-d interval that the majority of females took from emergence to the first bloodmeal, survival rate would have a similar value of 91.2%.

The parity rate of our colony females, 28%, is not valid for estimation of the survival rate. It may have been negatively biased by the small cage size or by low fertilization success in females exposed to males for only 24 h. Also, the use of the parity rate for the computation of the survival rate assumes a stable population, which is not the case with our colony because there were no new entries. The elevated survival rate of the colony females also probably is not realistic. These arguments suggest that the determination of vectorial capacity parameters from field observations, rather than laboratory observations, is preferred.

The vectorial capacity concept developed by Garrett-Jones (1964) after the initial modeling by Macdonald (1957) was directed toward the transmission of malaria by anopheline mosquitoes. Few studies have adapted this epidemiological entomological indicator to transmission of dengue or other arboviruses. Some of the parameters in the vectorial capacity formula have been previously estimated for *Ae. albopictus*, although vectorial capacity itself (the number of potentially infective bites by a local mosquito population that fed on one patient for one day) has not yet been reported.

Our computations of the vectorial capacity of *Ae. albopictus* from Macao were based on field and laboratory data and resulted in two values very different

from each other, 482 and 880. The excessively high vectorial capacity of 880 uses an artificially high survival rate from colony mosquitoes. The value of 482 is based on the parity rate from field mosquitoes and the length of the first gonotrophic cycle from laboratory mosquitoes, which was several days longer than that in published data, 4.6 + 2 d (Gubler and Bhattacharya 1971). The computation of the daily survival rate by using this published value and our observed parity rate would be 91.8%. This is similar to the rate of 88% (Hawley 1988) and calculations using these two rates result in vectorial capacity computations of 95 and 215, respectively.

Human daily biting rate was much lower in the dry season than in the rainy season, 94 opposed to 314, respectively. Correspondingly, the vectorial capacity had lower values. The vectorial capacity of any mosquito population is density-dependent, and this parameter is measured by the human-biting rate or incidence of bites per inhabitant per day (Garrett-Jones and Shidrawi 1969). The importance of this parameter has been highly stressed and constitutes a measurable and realistic indicator of human exposure to mosquito-borne diseases (Dye 1986, 1990). Our human daily biting rate should be considered a worst-case scenario, because the biting density measured in Coloane is definitely higher than that registered in the city of Macao, where most human population resides. If we projected the peak biting data from the study of the exophagic and endophagic behavior, by using the outdoor biting rates, assuming a similar diel pattern of activity and proportionate distribution of mosquitoes throughout the 24 h day, human daily biting rate in Taipa would be 248 mosquitoes/person/d; in Coloane, 111 mosquitoes/person/d; and in Macao, 39 mosquitoes/person/d. The corresponding vectorial capacities, by using 88% as daily survival rate (Hawley 1988), or our finding 91.8%, would be, respectively, 75 and 170 for Taipa, 34 and 76 for Coloane, and 12 and 27 for Macao city. An obvious difference can be seen between the three localities, and even in Coloane a wide range of variation can be expected in the same season. We should note that our calculation for vectorial capacity in the dry season uses some parameters derived from colony mosquitoes maintained in wet-season conditions and therefore may be biased in a positive direction. In the same way, with a lower environmental temperature in the dry season, vectorial capacity would be decreased due to a longer extrinsic incubation period.

The Macao climate seems to be optimal for the survival of *Ae. albopictus,* and thus the conditions could favor the transmission of dengue or other arboviral diseases. The high biting activity, parous rate, and anthropophily are all good indicators of this conclusion. The estimated values of the vectorial capacity of *Ae. albopictus* to dengue in Macao are high, thus this territory is very receptive to this arboviral disease.

Although at the time of this survey all the mosquitoes screened were negative for arboviruses, and only imported cases had been registered in Macao, the first notified dengue cases of autochthonous transmission were registered in 2001, when an outbreak with 1,418 cases occurred (Lim 2002, Chan and Chao 2002, Maia Trindade 2002). Dengue incidence has been increasing in Southeast Asia (Gibbons and Vaughn 2002, WHO 2002), and the current trend of global warming may further exacerbate its increase (Jetten and Focks 1997). The vectorial capacity values herein stated explain such outbreaks. Although we cannot assume that the entomological parameters at the time of the outbreak were similar to those we measured 3–4 yr before, the lower values for human biting rate and vectorial capacity in the city compared with the islands may explain why the outbreak did not result in even more cases.

The outbreak was mainly located in the "inner harbor" area of the city of Macao where boat houses were found infested with larvae. Other larval habitats identified included discarded rubber tires along the wharf, ornamental flower vases in and outside houses, water jars and barrels, and underground drains (Maia Trindade 2002). Such larval habitats had already been found in this area of town, in high numbers and with high breeding indexes (Ramos et al. 1997, Novo 2000).

Despite that some studies claim that there is little or no association between entomological population parameters and dengue cases, and although the minimum threshold of dengue vector density has not been scientifically defined (Service 1993, Reiter and Gubler 1997), the work described herein suggests that Macao was highly receptive to dengue, which the recent past came to demonstrate.

Acknowledgments

We thank the authorities in Macao, namely, the Health Centers, the Laboratory of Public Health, the Saint January Hospital, and Municipality of the Islands for cooperation. We thank all the members of the team that participated in this work, particularly the late Carl Mitchell (Centers for Disease Control and Prevention). For technical assistance, we thank Alan DuPuis (Centers for Disease Control and Prevention). This work was partially financed with Project Grant "*Aedes albopictus* in Macao: Systematics, Molecular Biology and Receptivity of the Territory to Dengue and Other Arboviroses" no. PRAXIS XXI/2/2.1/SAL/1388/95, coordinated by H.R., and also UPMM (Unidade Parasitologia e Microbiologia Médicas), FCT (Fundação para a Ciência e Tecnologia), Portugal, and FEDER (European Union). We also are indebted to Fundação Oriente and Fundação Macau, Lisbon, Portugal.

References Cited

Amerasinghe, F. P., and T. G. Ariyasena. 1991. Survey of adult mosquitoes (Diptera: Culicidae) during irrigation development in the Mahaweli Project, Sri Lanka. J. Med. Entomol. 28: 387–393.

Basio, R. G., and L. Santos-Basio. 1974. On Philippine mosquitoes. XIV. Biting cycles of some species in their natural forest habitat, with particular reference to *Aedes albopictus.* Kalikasan 3: 155–165.

Chan, K. L. 1971. Life table studies of *Aedes albopictus* (Skuse), pp. 131–144. *In* Sterility principles for insect

control or eradication. International Atomic Energy Agency, Vienna, Austria.

Chan, N. Y., and D.V.K. Chao. 2002. Dengue fever. H. K. Pract. 24: 395–400.

Clark, G. G., H. Seda, and D. J. Gubler. 1994. Use of the "CDC backpack aspirator" for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J. Am. Mosq. Control Assoc. 10: 119–124.

[CMI and IICT] Câmara Municipal das Ilhas e Instituto de Investigação Científica Tropical [eds.]. 1991. Macau-Catálogo de Plantas. Nomes científicos e vulgares em português, cantonense e inglês. CMI and IICT, Lisboa, Portugal.

Colless, D. H. 1959. Notes on the culicine mosquitoes of Singapore.VII. Host preferences in relation to the transmission of disease. Ann. Trop. Med. Parasitol. 53: 259–267.

Davidson, G. 1954. Estimation of the survival-rate of anopheline mosquitoes in nature. Nature (Lond.) 174: 792–793.

Detinova, T. S. 1963. Méthodes a appliquer pour classer par groupes dâge les Diptères prêsentant une importance médicale-Notamment certains vecteurs du paludisme. Org. Mond. de la Santé, Genéve, Switzerland.

Dias, J.A.T.S., L. F. Mendes, J. Crawford-Cabral, M. Pinheiro, L. M. Mesquitela, and L. P. Burnay. 1994. Resultados das missões de estudo realizadas em Macau pelo Centro de Zoologia do IICT. I - Introdução e natureza dos trabalhos efectuados. Sep. Garcia de Orta, Sér. Zool. 20: 1–30.

Dye, C. 1986. Vectorial capacity: must we measure all its components? Parasitol. Today 2: 203–209.

Dye, C. 1990. Epidemiological significance of vector-parasite interactions. Parasitology 101: 409–415.

Easton, E. R. 1994. Urbanization and its effects on the ecology of mosquitoes in Macau, Southeast Asia. J. Am. Mosq. Control Assoc. 10: 540–544.

Forattini, O. P. 1965. Entomologia médica, vol. 2. University of San Paulo, San Paulo, Brasil.

Freier, J. E., and D. B. Francy. 1991. A duplex cone trap for the collection of adult *Aedes albopictus.* J. Am. Mosq. Control Assoc. 7: 73–79.

Garrett-Jones, C. 1964. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. Nature (Lond.) 204: 1173–1175.

Garrett-Jones, C., and G. R. Shidrawi. 1969. Malaria vectorial capacity of a population of *Anopheles gambiae.* An exercise in epidemiological entomology. Bull. World Health Organ. 40: 531–545.

Garrett-Jones, C., P.F.L. Boreham, and C. P. Pant. 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971–78, with reference to the human blood index: a review. Bull. Entomol. Res. 70: 165–185.

Gibbons, R. V., and D. W. Vaughn. 2002. Dengue: an escalating problem. Brit. Med. J. 324: 1563–1566.

Gould, D. J., G. A. Mount, J. E. Scanlon, H. R. Ford, and M. F. Sullivan. 1970. Ecology and control of dengue vectors on an island in the gulf of Thailand. J. Med. Entomol. 7: 499–508.

Gubler, D. J., and N. C. Bhattacharya. 1971. Observations on the reproductive history of *Aedes* (*Stegomyia*) *albopictus* in the laboratory. Mosq. News 31: 356–359.

Hamon, J. 1963. Etude de lâge physiologique des femelles d'anophèles dans les zones traitées, de la région de Bobo-Dioulasso, Haute-Volta. Bull. World Health Organ. 28: 83–109.

Hao, W. Z. 1997. Árvores de Macau. Câmara Municipal das Ilhas, Macau.

Hassan, A. A., C. R. Adanan, and W. A. Rahman. 1996. Patterns in *Aedes albopictus* (Skuse) population density, host-seeking and oviposition behavior in Penang, Malaysia. J. Vector Ecol. 21: 17–21.

Hawley, W. A. 1988. The biology of *Aedes albopictus.* J. Am. Mosq. Control Assoc. Suppl. 1: 1–40.

Hien, D. S. 1976a. Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera, Culicidae). IV. The feeding of females. Acta Parasitol. Pol. 24: 27–35.

Hien, D. S. 1976b. Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera, Culicidae). V. The gonotrophic cycle and oviposition. Acta Parasitol. Pol. 24: 37–55.

Ho, B. C., K. L. Chan and Y. C. Chan. 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 3. Population fluctuations. Bull World Health Organ. 44: 635–641.

Jensen, T., O. R. Willis, T. Fukuda, and D. R. Barnard. 1994. Comparison of bi-directional fay, omni-directional, CDC and duplex cone traps for sampling adult *Aedes albopictus* and *Aedes aegypti* in North Florida. J. Am. Mosq. Control Assoc. 10: 74–78.

Jetten, T. H., and D. A. Focks. 1997. Potential changes in the distribution of dengue transmission under climate warming. Am. J. Trop. Med. Hyg. 57: 285–297.

Jumali, S., D. J. Gubler, S. Nalim, S. Eram, and J. S. Saroso. 1979. Epidemic dengue hemorrhagic fever in rural Indonesia. III. Entomological studies. Am. J. Trop. Med. Hyg. 28: 717–724.

Kirkwood, B. R. 1988. Essentials of medical statistics. Blackwell Scientific Publications, Oxford, United Kingdom.

Klowden, M. J., and H. Briegel. 1994. Mosquito gonotrophic cycle and multiple feeding potential: contrasts between *Anopheles* and *Aedes* (Diptera: Culicidae). J. Med. Entomol. 31: 618–622.

Knudsen, A. B. 1995. Global distribution and continuing spread of *Aedes albopictus.* Parassitologia 37: 91–97.

Lambrecht, F. L. 1971. Notes on the ecology of *Seychelles* mosquitoes. Bull. Entomol. Res. 60: 513–532.

Leitão, A. N. 1921. Tellurogie et climatologie médicales de Macao. Macao Station Climatique, Macao.

Lim, W. 2002. Dengue fever: where do we position ourselves? Newsletter, The Hong Kong Epidemiological Association 6: 3.

Macdonald, G. 1957. The epidemiology and control of malaria. Oxford University Press, London, United Kingdom.

Macdonald, W. W., and R. Traub. 1960. An introduction to the ecology of the mosquitoes of the lowland dipterocarp forest of Selangor, Malaya. Stud. Inst. Med. Res. 29: 79–109.

Maia Trindade, M. D. 2002. Dengue outbreak in Macao in 2001. Dengue Control Report by Macao Centre for Control and Prevention, Macao.

Mitchell, C. J. 1995. The role of *Aedes albopictus* as an arbovirus vector. Parassitologia 37: 109–113.

Newhouse, V. R., R. W. Chamberlain, J. F. Johnston, and W. D. Sudia. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. Mosq. News 26: 30–35.

Niebylski, M. L., H. M. Savage, R. S. Nasci, and G. B. Craig Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. J. Am. Mosq. Control Assoc. 10: 447–450.

Novo, M.T.L.M. 2000. Sobre a sistemática e ecologia larvar de *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) em Macau. Master's thesis, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal.

Parsons, R. E., T. J. Dondero, Jr., and C. W. Hooi. 1974. Comparison of CDC miniature light traps and human biting collections for mosquito catches during malaria

vector surveys in peninsular Malaysia. Mosq. News 34: 211–213.

Ramos, H. C. 1990. Seven new culicine records from Macao (Diptera, Culicidae). Bolm. Soc. Port. Entomol. IV-27: 325–340.

Ramos, H. C. 1994. Resultados das missões de estudo realizadas pelo Centro de Zoologia do IICT. V - Contribuição para o conhecimento dos culicíneos de Macau (Diptera, Culicidae). Garcia de Orta, Sér. Zool. 20: 77–112.

Ramos, H. C., H. Ribeiro, M. T. Novo, and E. R. Easton. 1997. Os Mosquitos de Macau (Diptera, Culicidae). SPEN, Lisboa, Portugal.

Ramos, H. C., H. Ribeiro, C. J. Mitchell, S. Baptista, M. L. Anselmo, M. T. Novo, C. Sousa, A.P.G. Almeida, E. R. Easton, and M. J. Pedro. 2002. Two new *Uranotaenia* records for Macau (Diptera: Culicidae). Garcia De Orta (Sér. Zool.) 24: 1–2.

Ramos, H. C., H. Ribeiro, C. J. Mitchell, M. T. Novo, S. Baptista, C. Sousa, A.P.G. Almeida, M. J. Pedro, E. R. Easton, and M. L. Anselmo. 2000. On *Coquilletidia crassipes,* a new record for Macau, with a key to adults of the subgenera and species groups of the genus (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 16: 66–70.

Reiter, P., and D. J. Gubler. 1997. Surveillance and control of urban dengue vectors, pp. 425–462. *In* D. J. Gubler and G. Kuno [eds.], Dengue and dengue hemorrhagic fever. CAB International, Wallingford, United Kingdom.

Ribeiro, H., and J. G. Janz. 1990. Exophagy and exophily in malaria vectors. Bull. Soc. Vector Ecol. 15: 185–188.

Rozeboom, L. E., and B. D. Cabrera. 1964. Filariasis in Mountain Provence, Luzon, Republic of the Philippines. J. Med. Entomol. 39: 18–28.

Savage, H. M., M. L. Niebylski, G. C. Smith, C. J. Mitchell, and G. B. Craig Jr. 1993. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. J. Med. Entomol. 30: 27–34.

Service, M. W. 1993. The role of ecology and ecological modeling in vector control. Bull Soc. Vector Ecol. 18: 85–98.

Simões, M. J., M. I. Próspero, and H. Ribeiro. 1995. Optimização da técnica ELISA "two-sites" utilizada na identificação de refeições sanguíneas de mosquitos. Rev. Portug. Doenças Infecc. 18: 225–229.

[WHO] World Health Organization. 2002. Dengue prevention and control. Wkly. Epidemiol. Rec. 77: 41–44.

Xue, R., and D. R. Barnard. 1996. Human host avidity in *Aedes albopictus:* influence of mosquito body size, age, parity, and time of day. J. Am. Mosq. Control Assoc. 12: 58–63.

Received 31 August 2004; accepted 8 February 2005.